Genetic Variation and Population Structuring in the Rosyside Dace, *Clinostomus funduloides,* in Ohio[1]

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ABSTRACT. The rosyside dace, although abundant throughout the eastern Appalachians, is listed as an endangered species in Ohio. It inhabits the headwater streams of south-central Ohio. Patterns of variation at four polymorphic loci (Adh, Ldh, Pgd, and Xdh) were analysed to determine the degree of interpopulation differentiation. Several characteristics (headwater resident, subject to annual stream drying, likely severe population crashes and flushes) of this species and its populations suggested that the rosyside dace should exhibit low intrapopulation variation and high interpopulation differentiation. The results were consistent with these predictions.

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INTRODUCTION

Species that are restricted to headwater streams tend to exhibit somewhat disjunct distributions locally, depending upon their tolerance of downstream conditions. If these headwater creeks are subject to annual periods of drying, populations inhabiting these waters will likely experience considerable fluctuations in size with some local extinctions and subsequent recolonizations. Populations may be reduced to a few individuals within isolated pools. Population genetic theory would predict that these populations should be characterized by low levels of intrapopulation variation (Nei et al. 1975). Genetic drift among these populations should result in relatively high levels of interpopulation differentiation.

The rosyside dace, *Clinostomus funduloides,* is a headwater fish species that is common throughout much of the eastern Appalachians and Tennessee (Lee et al. 1980). A small "finger" of distribution extends to south-central Ohio, where this species is considered to be endangered. In Ohio, the rosyside dace exhibits a very local distribution within the Scioto Brush, Sunfish and Little Scioto drainages, and in some of the small tributaries of Lawrence, Scioto, and Adams counties, which feed directly into the Ohio River (Rice and Phinney 1985).

Populations of the rosyside dace in Ohio were surveyed for allozyme variation to determine the extent of intra- and interpopulation variation. These data were used to address the following questions. Do these populations exhibit the patterns of genetic variation one might expect of a headwater species? Or, with the growing interest in "genetic management" of endangered species, does the level of genetic variation, as assayed by allozyme electrophoresis, indicate populations which, because of low variation, may be unable to respond in an evolutionary sense to changes in the environment?

METHODS AND MATERIALS

Forty localities were sampled. However, samples sufficient for electrophoretic analysis were obtained from only six localities (Fig. 1 and Table 1) in the Little Scioto River, Scioto Brush Creek, and Sunfish Creek during 1986. Fish were frozen on dry ice and stored at −90C until processed (two-three wks). For each individual, homogenates of muscle, liver, and eye tissue were subjected to cellulose acetate electrophoresis (Helena Laboratories) using stains adapted from Gauldie and Smith (1978) and Turner (1983). Thirty presumptive structural loci were surveyed: Alcohol dehydrogenase, Adh(2);

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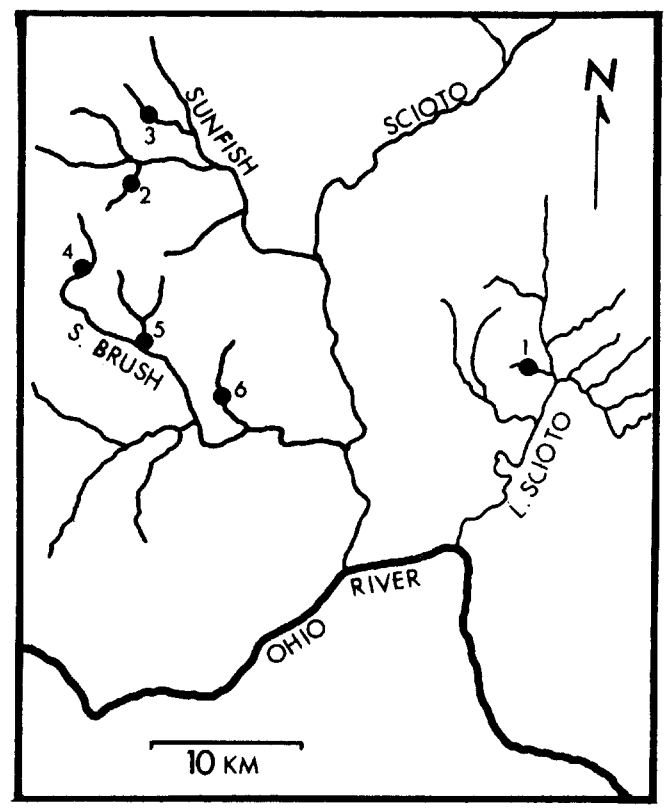

FIGURE 1. Map of locations of populations of *Clinostomus funduloides* used in the allozyme analysis. Population designations are as follows: 1, Buckhorn Creek, Scioto County; 2, Grassy Fork of Sunfish Creek, Pike County; 3, Morgan Fork of Sunfish Creek, Pike County; 4, Betty's Creek, Adams County; 5, Rarden Creek, Scioto County; 6, McCullough Run, Scioto County.

TABLE 1

Population designations, sample sizes, and genetic variability estimates for populations of C. funduloides *in Ohio. P_{gg} is the percent of loci polymorphic where the frequency of the alternate allele is >1%; H is the average individual heterozygosity.*

Locality	Designation	N	P_{gg}	H
Buckhorn Creek	1	24	.037	.009
Grassy Fork	2	14	.000	.000
Morgan Fork	3	10	.000	.000
Betty's Creek	4	13	.000	.000
Rarden Creek	5	11	.037	.009
McCullough Run	6	19	.111	.013

Alphaglycerolphosphate dehydrogenase, Agp; Acid phosphatase, Acp; Creatine kinase, Ck; Esterase, Est; Fumarate hydratase, Fh(2); Glutamate-oxaloacetate transaminase, Got(2); Glyceraldehyde phosphate dehydrogenase, Gpd(2); Isocitrate dehydrogenase, Idh; Lactate dehydrogenase, Ldh(3); Malate dehydrogenase, Mdh(2); Malic enzyme, Me; Muscle proteins, Mp(3); Phosphogluconate dehydrogenase, Pgd; Phosphoglucomutase, Pgm; Phosphohexose isomerase, Phi(2); Prolyldipeptidase, Pro; Superoxide dismutase, Sod; Tripeptidase (leucylglycylglycine), Trp; and Xanthine dehydrogenase, Xdh. Gels were run at room temperature for 30-45 min at 75V per gel using either a Barbital buffer (pH 8.6, Sigma Chemical) or Tris-Citrate (pH 6.1) (Turner 1983). Data analysis was performed with the BIOSYS-1 computer program (Swofford and Selander 1979).

RESULTS AND DISCUSSION

Sample sizes and standard measures of genetic variability for the six populations of *Clinostomus* are listed in Table 1. Sample sizes, although in some cases small, are sufficient to obtain relatively accurate estimates of heterozygosity and genetic distance (Gorman and Renzi 1979). Of the 30 loci surveyed, only four exhibited variation in at least one population (Table 2): Ldh-1, Adh-1, Pgd, and Xdh. A Chi-square analysis (Workman and Niswander 1970) revealed no significant departures from Hardy-Weinberg equilibrium.

The *Clinostomus* populations exhibited very low levels of genetic variation as compared with other genera of North American freshwater fishes (Buth 1986). Estimates of cyprinid heterozygosity (Avise 1977) averaged 0.052, markedly higher than 0.005 ± 0.002 (SE) observed among these populations of *Clinostomus* (Table 1). Although heterozygosity estimates for cyprinid genera other than *Notropis* are lower (0.036 ± 0.008; Buth 1986), they are still considerably higher than the estimates for *C. funduloides*. Although the cause and significance of these low levels of variation are unknown, such variation is consistent with what was proposed earlier for a headwater species.

The Nei genetic diversity measure (Nei 1975), which partitions the total gene diversity (H_t) into within (H_s) and between (D_{st}) subpopulation components, was used to assess the degree of interpopulation differentiation. This analysis indicated that approximately 86% of the total gene diversity was due to within-population variation; 14% was due to interpopulation differentiation ($H_t = 0.048$, $H_s = 0.042$, $D_{st} = 0.006$, $G_{st} = 0.139$). These results suggest that these populations, although not highly variable to begin with, do not show much interpopulation differentiation (Nei 1975). However, genic heterogeneity Chi-square analyses indicated significant ($P < .002$) heterogeneity among populations at Ldh-1 ($p < .001$) and Adh-1 and highly significant ($P < .001$) overall heterogeneity.

A dendrogram (UPGMA) was generated from the genetic distances (Fig. 2). This analysis generated three groups of populations, although the absolute distances were quite small (.000–0.26) and consistent with distances observed among conspecific populations of a variety of species. The Buckhorn Creek population was the most distinct, with Rarden Creek and McCullough Run comprising a second group. The other three populations displayed no genetic variation at 30 loci. Except for Betty's Creek, the dendrogram reflected the drainage relationships among these populations. Buckhorn Creek is in the Little Scioto drainage; Rarden Creek, McCullough Run and Betty's Creek are Scioto Brush tributaries; Grassy Fork and Morgan Fork are in the Sunfish Creek drainage.

The pattern of low intrapopulation variation is consistent with the predictions made for a headwater species subject to routine bottleneck episodes. If the headwater hypothesis is true, can it be predicted that many headwater species should show similar patterns of variation? Local extinctions owing to stream drying may be a common phenomenon for headwater species. Often, these streams must be recolonized from other populations that have survived the drying episode. (However, recent evidence on home range size [Hill and Grossman 1987] suggests that *C. funduloides* has in fact a rather small home range and is as sedentary as certain benthic forms.) Subsequent recolonization episodes may reestablish previous levels of genetic variation, thus minimizing local differentiation. In the case of *C. funduloides*, the ability to recolonize streams that have experienced extinctions may be dramatically reduced because of deteriorating conditions in many of the streams in that area. Recently documented localities (from Rice and Phinney 1985) failed to yield specimens of *C. funduloides* in 1986. The rosyside dace seems to require clear, rocky streams, free from siltation. Increased disturbance in this region has apparently made many of these streams unfit for this species. Buckhorn Creek and McCullough Run, the largest and possibly the most stable populations, were clearly the most pristine and also possessed the highest heterozygosities.

Finally, although the distribution of *C. funduloides* in Ohio is quite restricted and local, it is not difficult to find and in several streams is quite abundant. It's place on the state endangered species list may be open to some ques-

TABLE 2

Allele frequencies for populations of Clinostomus funduloides *from Ohio. See Table 1 for population designations.*

Locus		Population 1	2	3	4	5	6
LDH-1	a	.750	1.000	1.000	1.000	1.000	1.000
	b	.250	.000	.000	.000	.000	.000
ADH-1	a	1.000	1.000	1.000	1.000	.875	.842
	b	.000	.000	.000	.000	.125	.158
PGD	a	1.000	1.000	1.000	1.000	1.000	.947
	b	.000	.000	.000	.000	.000	.053
XDH	a	1.000	1.000	1.000	1.000	1.000	.974
	b	.000	.000	.000	.000	.000	.026

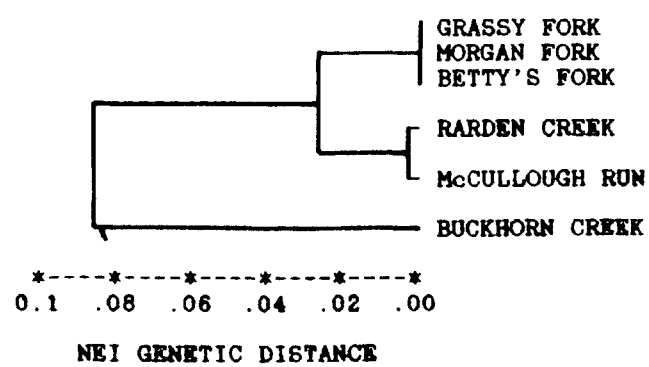

FIGURE 2. Dendrogram (UPGMA) of Nei genetic distances for populations of *Clinostomus funduloides*.

tion. Clearly, however, it should at least be considered a species of special concern whose status should be routinely monitored and steps taken to insure suitable habitat.

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